Exogenous porcine somatotropin administered to late pregnant gilts alters liver and muscle functionalities in pig foetuses

Florence Gondret, Isabelle Louveau, P Langendjik, Chantal Farmer

Animal 17 (2023) 100691

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Exogenous porcine somatotropin administered to late pregnant gilts alters liver and muscle functionalities in pig foetuses

F. Gondret [a,*], I. Louveau [a], P. Langendjik [b], C. Farmer [c]

[a] PEGASE, INRAE, Institut Agro, 35590 Saint-Gilles, France
[b] Trouw Nutrition Research & Development, Stationsstraat 77, Amersfoort, The Netherlands
[c] Agriculture and Agri-Food Canada, Sherbrooke R & D Centre, 2000 College, Sherbrooke (QC) J1M 0C8, Canada

ARTICLE INFO

Article history:
Received 20 May 2022
Revised 24 November 2022
Accepted 29 November 2022
Available online 7 December 2022

Keywords:
Carbohydrate metabolism
Energy status
Gene expression
Piglet
Somatotropin

ABSTRACT

Neonatal maturity depends on the maternal capacity to provide nutrients for foetal growth. This study aimed to investigate the effects of systemic administration of recombinant porcine somatotropin (**pST**), one of the main regulators of growth and metabolism, to pregnant gilts during late gestation on circulating nutrients and expression levels of genes in liver and skeletal muscle of their 110-day-old foetuses. Gilts received either daily injections of sterile water (control [**CTL**] group, n = 15) or of 5 mg of pST (pST group, n = 17) from days 90 to 109 of gestation. At day 110 postconceptus, pairs of foetuses (one of small and one of average size within a litter) were selected. Circulating fructose concentrations were greater, but circulating concentrations of urea were lower in pST than in CTL foetuses. Expression levels of genes involved in carbohydrate and lipid metabolism were more affected by pST treatment in liver than in muscle. Hepatic molecular changes suggest an inhibition of energy-consuming processes (glycogen and lipid biosynthesis) and the activation of energy-producing pathway (mitochondrial oxidation) in pST compared to CTL foetuses. Expression levels of some genes involved in intracellular degradation of proteins were greater in the liver of pST foetuses, and combined with lower uremia, this suggests a higher utilisation of protein sources in pST foetuses than in CTL foetuses. In muscle, molecular changes were mainly observed in the IGF-insulin axis. Altogether, pST-treated gilts seem to have a greater ability to support foetal liver development by the reorientation of energy and protein metabolism.

Implications

To reduce the early mortality of piglets in sows, solutions to improve physiological maturity of piglets just before birth are needed. The IGF axis is the main regulator of growth and metabolism in mammals. This study brings new data on the consequences of the modulation of somatotropin and IGF axis in gestating sows on the expression levels of some genes related to protein or energy metabolism and cell development in their foetuses. The potentially beneficial effects observed on foetal liver development can prepare neonatal metabolism to better adapt to the postnatal environment. Nutritional ways to promote these pathways could be further reasoned.

Introduction

In modern pig production, perinatal death averages 20% of the piglets, with important economic and ethical consequences. Physiological maturity is one of the most important factors influencing piglet survival from birth to weaning (Leenhouwers et al., 2002). Genetic selection for sow productivity during the last decades has led to a large increase in litter size (Quesnel et al., 2008), which increased the metabolic demands of the sow during the last third of gestation and the immaturity of piglets around birth (Canario et al., 2007). Approximately 60% of uterine energy deposition in sows occurs during the last 30 days of gestation (Noblet et al., 1990), a period during which the BW of the foetuses almost doubles (Hill and Mahan, 2016). The maturity of the neonate which is determined by the degree of tissue development is thus dependent on the availability of maternal nutrients to support foetal growth and on the nutrient uptake per foetus. Circulating concentrations of blood variables such as albumin, fructose and IGF-I (Canario et al., 2006; Gondret et al., 2018) and expression levels of metabolic genes in foetal tissues (Gondret et al., 2018) differed with the degree of foetal development. Especially, spontaneous intrauterine growth restriction was shown to alter the IGF system in adipose tissue and skeletal muscle of pig foetuses (Gondret et al., 2013; Perruchot et al., 2015).

* Corresponding author.
E-mail address: Florence.gondret@inrae.fr (F. Gondret).

https://doi.org/10.1016/j.animal.2022.100691
1751-7311/

The somatotropin (**ST**)-IGF axis is the main regulator of growth and metabolism in mammals, and during pregnancy, ST has anabolic and metabolic effects both directly via the GH-receptor (**GHR**) and indirectly via stimulation of IGF-I production at multiple target tissues (Kaur et al., 2021). Increased placental expression of human *IGF1* gene in an intrauterine growth restricted-induced mouse model was able to compensate for foetal growth (Abd Ellah et al., 2015) through the up-regulation of glucose transport mechanism (Jones et al., 2013). Adenoviral-mediated increased delivery of the human *IGF1* gene also counteracted spontaneous intrauterine growth restriction in rabbits (Keswani et al., 2015). Systemic administration of IGF-I in pregnant guinea pigs increased the placental uptake and transfer of nutrients, circulating levels of nutrients in the foetus, and foetal BW (Sferruzzi-Perri et al., 2006). All these examples suggest that increasing circulating IGF-I during gestation may improve the tissue development of foetuses. Recently, Farmer and Langendijk (2019) showed that gilts injected with recombinant porcine somatotropin (**pST**) during late gestation had greater circulating concentrations of IGF-I, insulin and glucose, together with lower circulating concentrations of urea. Although pST and IGFs do not cross the placenta, these changes may affect the physiological maturity of foetuses via a direct effect on the placenta or through changes in metabolic status of gilts that can modify nutrient availability for the uterine-placenta units.

The aim of this study was to investigate the effects of pST treatment of pregnant gilts during late gestation on expression levels of some genes related to protein or energy metabolism and cell development in liver and skeletal muscle collected in their 110-day-old foetuses. Because previous studies showed that the effects of maternal pST treatment on foetal growth may depend on their BW quartile (Rehfeldt et al., 2001), foetal tissues were sampled in small- and average-sized categories of piglets in each litter. Preliminary data have been presented in the International Symposium on Energy and Protein Metabolism and Nutrition (Gondret et al., 2022).

Material and methods

Experimental design

The experimental design was fully described by Farmer and Langendijk (2019). Briefly, 32 Yorkshire × Landrace gilts were bred via artificial insemination using pools of semen from Duroc boars. All gilts received a conventional gestation diet with feeding levels adjusted to BW from mating to day 89 of gestation and then received 2.5 kg of feed from day 90 onwards. On day 89 of gestation, gilts were separated into two groups: control (**CTL**, n = 15) or pST-treated (pST, n = 17). Control gilts received a daily injection of 1 mL of sterile water, whereas treated gilts received daily injections of 5 mg of pST (Reporcin, Zamira Life Sciences Pty Ltd, Knoxfield, Australia) diluted in 1 mL of sterile water from days 90 to 109 of gestation. All gilts were euthanised on day 110 ± 1 postconception. The uterus was removed, and foetuses were counted, sexed and weighed.

Blood and tissue sampling in foetuses

In each litter, two foetuses of the same sex (16 females and 14 males in CTL group, 16 females and 18 males in pST group) were selected to represent small- (0.89 kg ± 0.03 kg) or average-sized (1.18 kg ± 0.03 kg) categories. Foetuses (n = 64) were removed from the uterus 5–10 min after the gilt was euthanised and exsanguinated to ensure that they were already dead. For each foetus, blood was collected into Vacutainer tubes without anticoagulant (Becton Dickinson, Franklin Lakes, NJ, USA) and left at room temperature for 3 h, stored overnight at 4 °C, and centrifuged for 12 min at 1 800*g* at 4 °C the following day; serum was then harvested. Within 15 min after death, the liver was excised and weighed, and collected samples (approximately 2 g) were cut into small pieces and frozen in liquid nitrogen. At the same time, the *Longissimus* muscle was excised from the dorsal side and transversally cut; the whole section (400–700 mg) was divided into small pieces and frozen in liquid nitrogen. All tissue samples were kept at −75 °C until analysis.

Circulating plasma metabolites and hormones

Glucose, fructose and free fatty acids (**FFAs**) were chosen as indicators of energy metabolism, and albumin and urea were chosen to represent protein metabolism. Blood parameters were analysed using commercial kits on a Konelab analyzer 20i (Thermo Fisher Scientific). Suppliers were BioMérieux (Marcy l'Etoile, France) for the Glucose RTU and Albumin kits, Thermo Electron (Cergy-Pontoise, France) for the Fructose kit, Wako Chemicals GmbH (Neuss, Germany) for FFA (NEFA-HR2 kit) and Thermo Fisher Scientific (Courtaboeuf, France) for urea. All assays had CV less than 5%.

Gene expression

Total RNA was extracted from liver and muscle, using previously described methods (Vincent et al., 2015). Gene expression levels were studied by real-time quantitative PCR. Succinctly, first-strand complementary DNA was synthetised from 1 μg of total RNA using High Capacity RNA to DNA Kit (Applied Biosystems, Foster City, USA). Primers were designed from porcine sequences available in Ensembl or NCBI databases using Primer Express® v3.0 software (Applied Biosystems) or Primer Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast). Information on the selected genes is provided in Supplementary Table S1. The gene expression levels were measured using a SmartChip Real-time PCR system (Wafergen/Takara) available at the EcogenO Platform (OSUR, Rennes, France). This SmartChip nanowell system makes it possible to perform up to 5 184 reactions per run with 100 nL final volume for each well. Amplification reactions were carried out with a final complementary DNA concentration of 2 ng/μL and a primer concentration of 500 nM dispensed using the WaferGene SmartChip Multisample Nanodispenser. Amplification conditions were as follows: 5 min at 95 °C followed by 45 cycles of 10 sec at 95 °C, 30 sec at 60 °C and 30 sec at 72 °C, followed by 30 sec at 95 °C and 30 sec at 60 °C. The results were analysed using SmartChip qPCR Software (v 2.8.6.1). For a subset of genes having low expression levels in one of the tissues, real-time quantitative PCR was also performed using the Master Mix Fast Sybr Green (Applied Biosystems) and a StepOneplus Real-time PCR system (Applied Biosystems) and amplification program as follows: 50 °C–2 min, 95 °C-20 sec followed by 40 cycles at 95 °C–3 sec and 60 °C–30 sec. Specificity of the amplification products was checked by dissociation curve analysis. A normalisation factor (**NF**), one for the liver and one for the muscle, was calculated as the geometric average of *PPIA* and *RPL4* that were the most stable genes in these conditions as stated by GeNorm algorithm (Vandesompele et al., 2002). The normalised expression level (**N**) of each gene was calculated according to the formula $N = E - \Delta Cq$ (sample-calibrator)/NF, where efficiency (**E**) is calculated from the slope of calibration curve, **Cq** is the quantification cycle, and the calibrator is a pool of eight samples. For the studied genes, E was between 1.60 and 2.23 (1.78 on average).

Statistics

Data were analysed by analysis of variance using the GLM procedure of SAS (SAS, Cary NC, USA). The model included the fixed effects of maternal treatment (CTL or pST), class of foetal BW (small or average) and the interactions between maternal treatment and class of foetal BW. Difference was considered significant at $P \leq 0.05$. $0.05 < P \leq 0.10$ was discussed as trend.

Results

At day 110 of gestation, litter size averaged 13.6 and 12.1 piglets for pST and CTL gilts (Table 1) and was not affected by pST treatment ($P > 0.10$). Mean foetal BW in the litters did not differ between maternal treatments ($P > 0.10$), with values of 1.06 ± 0.17 kg and 1.14 ± 0.16 kg for pST and CTL gilts, respectively. According to the experimental design, the mean BW of the selected foetuses differed ($P < 0.001$) between categories. There was no significant effect of maternal treatment with pST on BW of the selected foetuses (Table 1).

Circulating concentrations of metabolites in foetuses

There were no interaction effects between treatment and class of foetal BW on the circulating concentrations of metabolites in the selected pairs of foetuses. Irrespective of the category of foetal BW, circulating concentrations of fructose were greater ($P = 0.001$) in foetuses of pST gilts than in foetuses of CTL gilts, whereas circulating urea concentrations were lower ($P < 0.001$) in pST foetuses than in CTL foetuses (Table 2). Circulating concentrations of glucose, FFA and albumin did not differ between pST and CTL foetuses. Irrespective of maternal treatment, there were no differences in circulating concentrations of metabolites between categories of foetal BW, with the noticeable exception of FFA concentrations which tended to be greater ($P < 0.10$) in small foetuses than in their average-sized littermates.

Expression levels of genes related to energy metabolism in liver and muscle

There were no interaction effects between maternal treatment and class of foetal BW on the expression levels of genes in liver and muscle of the selected foetuses. Expression levels of genes involved in carbohydrate and lipid metabolism were generally more affected by pST treatment in liver than in muscle (Supplementary Table S2). As shown in Fig. 1A, the facilitated glucose transporter member 1 (*SLC2A1*) responsible for basal uptake of glucose and other hexoses was up-regulated (+16%, $P < 0.01$) in liver of pST foetuses as compared with controls. The hepatic mRNA level of glycogen synthase kinase 3 beta (*GSK3B*), acting as a negative regulator in the hormonal control of glucose homeostasis by inactivating glycogen synthase and hence glycogen synthesis, was greater (+8%; $P = 0.01$) in pST foetuses than in CTL foetuses. The gene expression of catalytic subunit alpha1 (*PRKAA1*) of the 5′-AMP-activated protein kinase (**AMPK**), a cellular energy sensor that activates energy-producing pathways such as fatty acid oxidation and inhibits energy-consuming processes such as protein, carbohydrate and lipid biosynthesis, also tended ($P < 0.10$) to be up-regulated in the liver of foetuses from pST gilts. The peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PPARGC1*), that coordinates the expression of various genes involved in glucose and fatty acid metabolism, was up-regulated in liver of pST foetuses as compared with CTL foetuses. Accordingly, genes participating in the intracellular transport of long-chain fatty acids and their acyl-CoA esters by fatty acid binding protein-3 (***FABP3***) and regulating mitochondrial uptake for their subsequent beta-oxidation by carnitine palmitoyltransferase I (***CPT1A***) had a greater expression level (+16% on average) in pST than CTL foetuses. On the other hand, fatty acid synthase (***FASN***), a gene that catalyses biosynthesis of long-chain saturated fatty acids from carbohydrates, was down-regulated (−18%, $P = 0.01$) in the liver of pST foetuses as compared with CTL foetuses.

In the *Longissimus* muscle, the catalytic subunit alpha of AMPK (*PRKAA1*) was down-regulated by pST maternal treatment (Fig. 2A). However, the gene encoding the gamma subunit of AMPK (*PRKAG3*) which is dominantly expressed in skeletal muscle had a similar expression level in both groups of foetuses. The mRNA levels of *FABP3* and *CPT1A* were ($P < 0.05$) or tended to be ($P < 0.10$) greater in the muscle of pST foetuses as compared with CTL foetuses. The other studied genes involved in fatty acid oxidation (*CPT1B, PPARGAC1*), in lipolysis (the adipocyte type of FABP [*FABP4*] and the hormone-sensitive type of lipase *[LIPE]*), and in fatty acid synthesis (*FASN*) did not differ between the two maternal treatments.

The category of foetal BW was associated with altered expression levels of more genes in muscle than in liver tissue (Supplementary Table S2). Muscle *GSK3B* mRNA levels were greater in small foetuses than in their average-sized littermates ($P \leq 0.05$), whereas muscle isozyme of the phosphofructokinase (*PFKM*) involved in the first committing step of glycolysis had a lower expression level in small than in average-sized foetuses (Fig. 3). Expression levels of *CPT1A* (liver isoform) were greater in small foetuses than in their average-sized littermates (Supplementary Table S2), whereas expression levels of *CPT1B* (muscle isoform) did not differ between the two classes of foetal BW (Fig. 3).

Expression levels of genes related to protein metabolism in liver and muscle

In the liver, mRNA level of the component of the 26S proteasome (*PSMD1)* involved in the ATP-dependent degradation of ubiquitinated proteins was higher (+8%, $P = 0.01$) in foetuses of pST gilts

Table 1
Litter traits in control and treated gilts.

	Treatment			
	CTL	pST	RMSE	*P*-value
Litter	15	16		
Size (n)	12.1	13.6	3.6	0.13
(Min; Max)	(6; 17)	(5; 20)	–	–
Mean foetal BW (kg)	1.14	1.06	0.17	0.34
(Min; Max)	(0.45; 1.66)	(0.37; 1.76)	–	–
Selected piglets				
Mean foetal BW (kg)	1.04	1.02	0.17	0.53
(Min; Max)	(0.63; 1.38)	(0.70; 1.55)	–	–

Abbreviations: CTL = controls (injected with sterile water); pST = treated with 5 mg of porcine somatotropin from days 90 to 109 of gestation.

Table 2
Circulating concentrations of metabolites in 110-day-old pig foetuses.

	Treatment[1]		BW category[2]			*P*-value		
Metabolites	CTL	pST	Average	Small	RMSE	T	W	T × W
Glucose (mmol/L)	3.46	3.63	3.79	3.30	1.48	0.66	0.20	0.47
Fructose (mmol/L)	4.08	4.97	4.69	4.36	1.02	0.001	0.20	0.87
FFA (µmol/L)	40.0	39.2	37.9	41.4	7.67	0.64	0.06	0.62
Albumin (g/L)	5.87	6.01	6.09	5.81	1.08	0.61	0.31	0.75
Urea (mg/L)	295	152	218	229	50	<0.001	0.35	0.45

Abbreviations: CTL = controls (injected with sterile water); pST = treated with 5 mg of porcine somatotropin from days 90 to 109 of gestation; FFAs = free fatty acids; T = maternal treatment; W = category of foetal BW; T × W = interaction between maternal treatment and category of foetal BW.
[1] N = 15 for CTL gilts and N = 17 for pST gilts.
[2] Pairs of piglets from small- or average-sized BW were selected within each litter.

as compared to foetuses of CTL gilts (Fig. 1B). In addition, hepatic mRNA abundance of the lysosomal cysteine protease (*CTSB*) playing major roles in the intracellular degradation of proteins was 12% higher ($P < 0.05$) in pST than in CTL foetuses. Treatment-associated difference in the hepatic mRNA levels of the cysteine protease (*CTSD*) involved in the proteolytic activation of growth factors did not reach statistical significance ($P = 0.11$). Finally, hepatic expression levels of the ubiquitous enzyme calpain 1 (*CAPN1*) catalysing the non-lysosomal limited proteolysis of substrates, and of the ubiquitin-conjugating enzyme E2 Q1 (*UBE2Q*), a gene involved in ubiquitination of proteins for degradation, did not differ between pST and CTL foetuses. With regard to protein synthesis, expression levels of the eukaryotic translation initiation factors (**EIFs**) such as *EIF1*, *EIF3A* and *EIF4B* in the liver did not differ between pST and CTL foetuses. The raw weight of the liver did not differ between maternal treatments (30.7 + 1.2 g on average, Fig. 1B). When expressed relative to foetal BW, the liver was heavier in pST foetuses than in CTL foetuses ($P < 0.05$).

In the *Longissimus* muscle, none of the studied genes involved in protein degradation or protein synthesis were affected by maternal pST treatment (Fig. 2B). There were no differences between categories of foetal BW in the expression levels of genes involved in protein metabolism, except *EIF1* that tended ($P < 0.10$) to have a lower expression level in muscle of small foetuses as compared with average-sized littermates (Supplementary Table S3).

Expression levels of genes related to cell development and growth in liver and muscle

In both liver and muscle, the expression levels of genes of the IGF-binding protein (**IGFBP**) family did not differ between maternal treatments, except *IGFBP5* which was more expressed in the liver of pST foetuses as compared to CTL foetuses (+21%; $P < 0.01$; Supplementary Table S4) within the average-sized category of foetuses. In liver, there was no difference in expression levels of genes related to the IGF-insulin axis. In *Longissimus* muscle, expression levels of *IGF1* and *RIGF1* tended to be lower ($P \leq 0.10$) in pST than in CTL foetuses (Fig. 3) and the receptor for insulin (*RINS*) was down-regulated ($P < 0.05$) in the muscle of pST foetuses. Moreover, the paired box transcription factor (*PAX7*), a gene playing critical roles during foetal development, and the mitogen-activated protein kinase (*MAPK3*) that encodes an extracellular signal-regulated kinase that regulates cellular processes, were down-regulated ($P < 0.05$) in the muscle of pST compared with CTL foetuses.

Irrespective of maternal treatment (Supplementary Table S4), genes involved in the regulation of cell development and growth were differentially expressed between categories of foetal BW. In liver, the expression level of *IGFPB1* was greater ($P \leq 0.05$) in small foetuses than in average-sized foetuses. In *Longissimus* muscle, *IGF1* tended to be repressed ($P < 0.10$) in small foetuses, whereas its receptor (*RIGF1*) had a greater expression level ($P = 0.05$) in small foetuses compared with their average-sized littermates. In addition, the expression level of Delta-Like Non-Canonical Notch Ligand 1 (***DLK1***) in muscle was markedly higher (+67%; $P < 0.001$) in small foetuses than in their average-sized littermates.

Discussion

In the current study, pST was administrated to gilts during the last month of gestation (90–110 days; gestation term: 114 days), a period when foetal growth is maximal. Although the BW of foetuses from pST-treated and control gilts at 110 days of gestation did not differ statistically (when corrected for litter size), the molecular profiles in liver and skeletal muscle and the biochemical traits in blood of foetuses were significantly affected by maternal treatment. The influence of pST was previously reported to be more pronounced on growth and body composition of small foetuses than in their median- and heavy-BW littermates when pST was injected to sows during early gestation (Rehfeldt et al., 2004). Herein, there were almost no interaction effects between maternal treatment and categories of foetal BW (small/average) for the studied traits. The difference in BW between selected small- and medium-sized categories of pig foetuses was however smaller in this study than in other studies (Gondret et al., 2013; Perruchot et al., 2015) addressing specifically intrauterine growth restriction in pig foetuses.

The finding of a heavier liver (when corrected by foetal BW) in pST foetuses compared with controls at 110 days of gestation agrees with the study of Gatford et al. (2000) showing that liver weight in foetuses at day 51 of pregnancy increased with increasing doses of pST administered to the sow during the second quarter (25–51 days) of pregnancy. In contrast, Kveragas et al. (1986) indicated that liver weight (corrected by birth BW) did not differ between neonates from sows treated with pST 21 days prior to farrowing and neonates from untreated sows. Maternal pST treatment can improve placental function and/or increase the circulating levels of metabolites in maternal plasma, and these changes can facilitate the transfer of nutrients from the dam to foetuses and thus influence foetal tissue development. In the current study, glucose concentrations in foetal blood collected at day 110 of gestation did not differ between the two treatments, despite the greater blood glucose concentrations reported in pST-treated gilts compared to control gilts at days 96, 103 and 109 of gestation in the same experimental design (Farmer and Langendijk, 2019). Gatford et al. (2000) showed that foetal plasma glucose concentrations changed in a quadratic fashion with the dose of pST injected into pregnant gilts, and they did not report any significant associations between foetal plasma glucose concentrations and maternal plasma glucose concentrations at particular gestation points. This can explain the lack of difference in foetal glycaemia between pST and CTL foetuses in our study. Conversely, we found greater

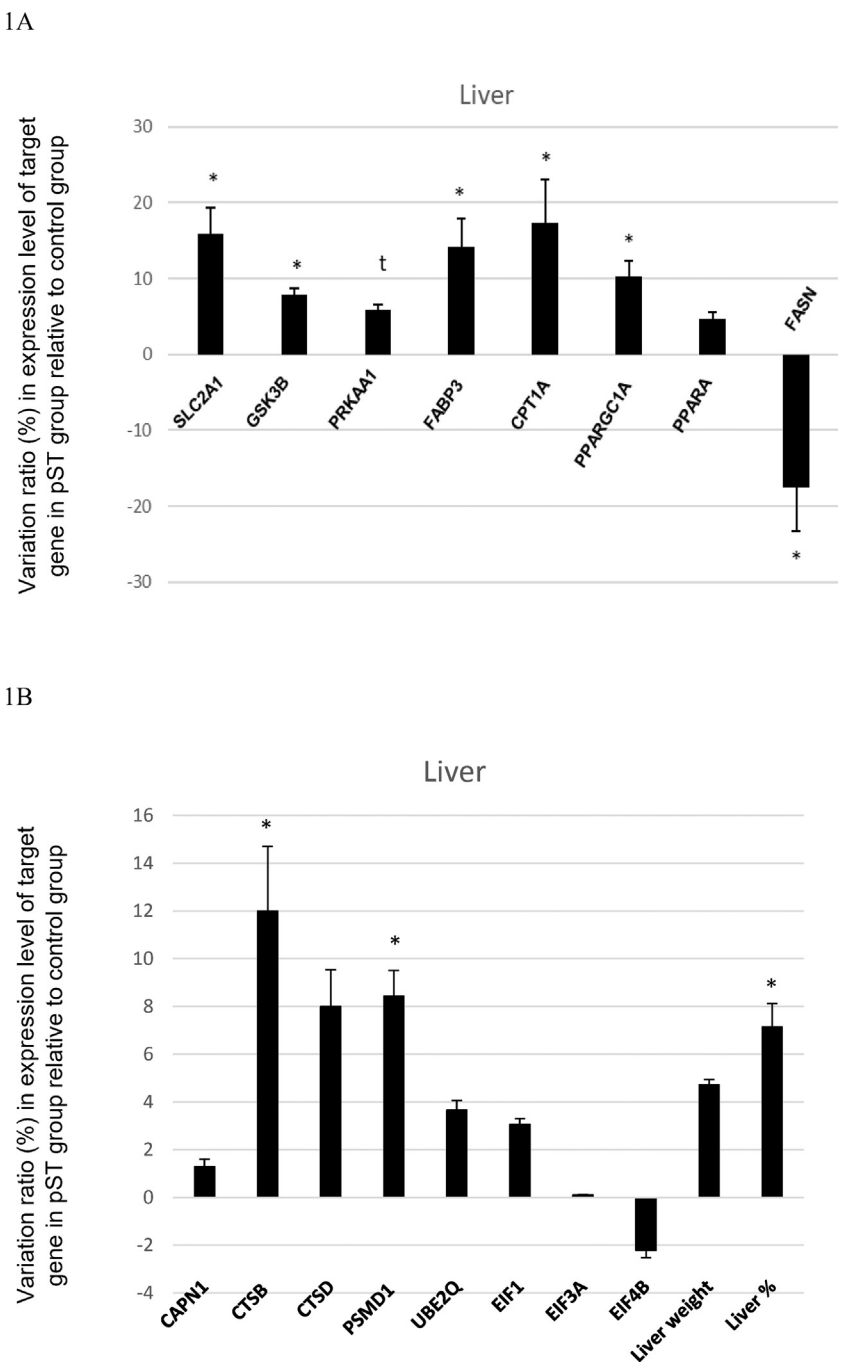
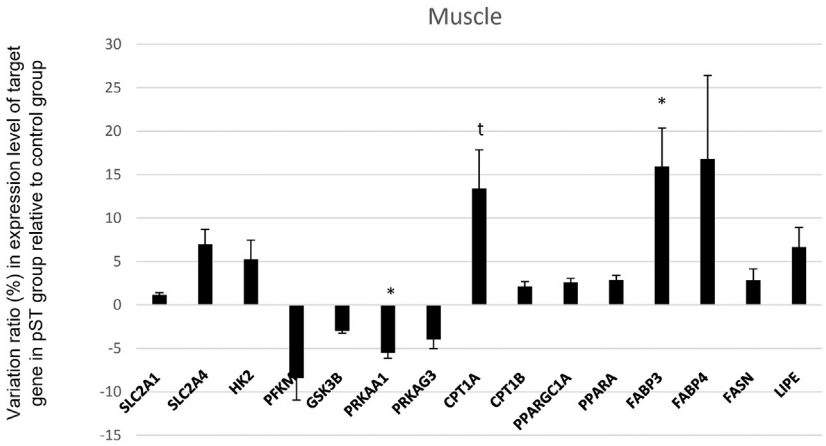


Fig. 1. Hepatic expression levels of genes involved in energy and protein metabolism in foetuses from control or pST-treated gilts. Foetuses of gilts injected with sterile water (CTL) or 5 mg of pST were collected at 110 days of gestation. The liver was immediately excised from pairs of foetuses (one small, one of average BW) and prepared for molecular analysis. Target genes involved in energy (1A) or protein (1B) metabolism were compared for the effects of maternal treatment, category of foetal BW, and the interaction between maternal treatment and foetal BW category. The %variation ratio (mean + SEM) in expression level of each target gene was shown for pST foetuses relative to controls. When a gene was down-regulated by the pST treatment, the value was preceded by a minus sign. Liver weight was also indicated as raw weight or expressed relative to foetal BW. $^{*}P \leq 0.05$, $^{t}0.05 < P \leq 0.10$. Abbreviations: CTL = controls; pST = porcine somatotropin.

plasma concentrations of fructose in pST foetuses compared to control foetuses at day 110 of gestation. The pig conceptus is fructogenic so that a substantial portion of glucose transferred from the dam to the foetus can be converted into fructose within the placenta (Vallet et al., 2014). Fructose is the most abundant hexose sugar in foetal blood and other foetal fluids and it can be converted to fructose-1-phosphate by ketohexokinase (also known as fructokinase), an enzyme that is present notably in the liver but that is also expressed in muscle (Diggle et al., 2009). This pathway bypasses the major glycolytic checkpoint at the level of phosphofructokinase. Fructose can contribute to various metabolic pathways, such as hexosamine biosynthesis pathway and one-carbon metabolism. Because fructose is an intermediate product in the synthesis of glucosamine involved in the regulation of cell proliferation, the greater circulating concentrations of fructose in pST foetuses may be a key for promoting cell proliferation and subsequent tissue growth. In liver, various genes involved in glucose metabolism then showed an altered expression in foetuses from pST gilts compared to foetuses from control gilts. The liver contains *SLC2A1* to regulate glucose and fructose uptake from circulating blood.

2A



2B

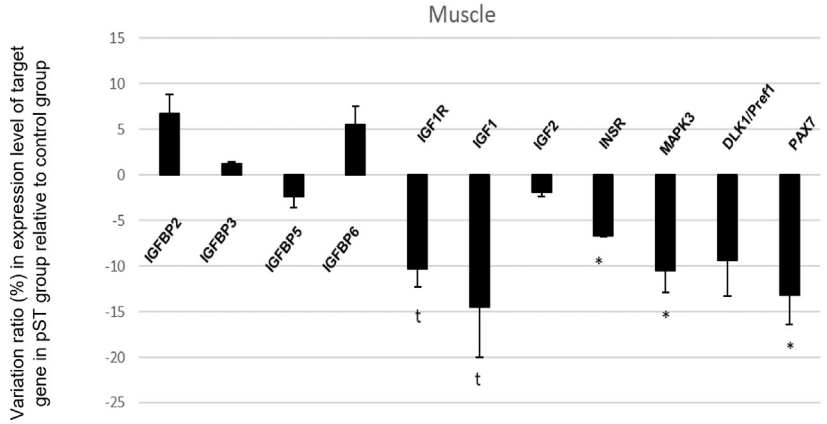


Fig. 2. Muscle expression levels of genes involved in energy metabolism or regulation of cell development and growth in foetuses from control or pST-treated gilts. Foetuses of gilts injected with sterile water (CTL) or 5 mg of porcine pST were collected at 110 -days of gestation. The *Longissimus* muscle was immediately excised from pairs of foetuses (one small, one of average BW) and prepared for molecular analysis. Expression levels of target genes involved in energy metabolism (2A) or the regulation of cell development and growth (2B) were compared for the effects of maternal treatment, category of foetal BW, and the interaction between maternal treatment and BW category. The %variation ratio (mean + SEM) in expression level of each target gene was shown for pST foetuses relative to controls. When a gene was down-regulated by the pST treatment, the value was preceded by a minus sign. $^{*}P \leq 0.05$, $^{t}0.05 < P \leq 0.10$. Abbreviations: CTL = controls; pST = porcine somatotropin.

Expression level of *SLC2A1* was greater in liver of pST foetuses, which may favour the transport of sugars in hepatocytes from those foetuses as compared with controls. This change was associated with a molecular signature towards an inhibition of energy-consuming processes such as glycogen (increased expression level of *GSK3B*) and lipid biosynthesis (decreased expression level of *FASN*), but the activation of energy-producing pathways such as fatty acid oxidation (higher expression levels of *FABP3* and *CPT1A*), in the liver of pST compared with control foetuses. Together with the greater mRNA levels of alpha1-AMPK (*PRKAA1*), a crucial cellular energy sensor (Towler and Hardie, 2007), and of *PPARGC1* (PGC-1), a coactivator coordinating multiple aspects of the fasted response in liver (Puigserver and Spiegelman, 2003), these molecular changes suggest reallocation of energy in the liver of pST foetuses. Because embryonic development is heavily dependent on the metabolic status, this activation of hepatic energy-producing pathways in pST foetuses may have sustained the somatic development of the liver (relative to foetal BW) as compared with control foetuses. This observation matches the preferential development of internal organs relative to other organs reported in foetuses from pST-treated sows during early pregnancy as compared with controls (Rehfeldt et al., 2004). This increase could be due to higher protein accretion, an hypothesis that is supported by the lower uremia observed in the pST-treated group of foetuses than in controls. Whether this was also accompanied by differences in the amount and type of energy reserves, due to the repression of genes involved in energy-consuming processes in pST foetuses, deserves further studies. Finally, the liver may also catabolise fatty acids to produce 3-carbon substrates for gluconeogenesis as an adaptive response for supplying glucose to any other glucose-consuming foetal tissues such as skeletal muscle (Thorn et al., 2011). Significant gluconeogenesis has not been detected in foetal piglet in the

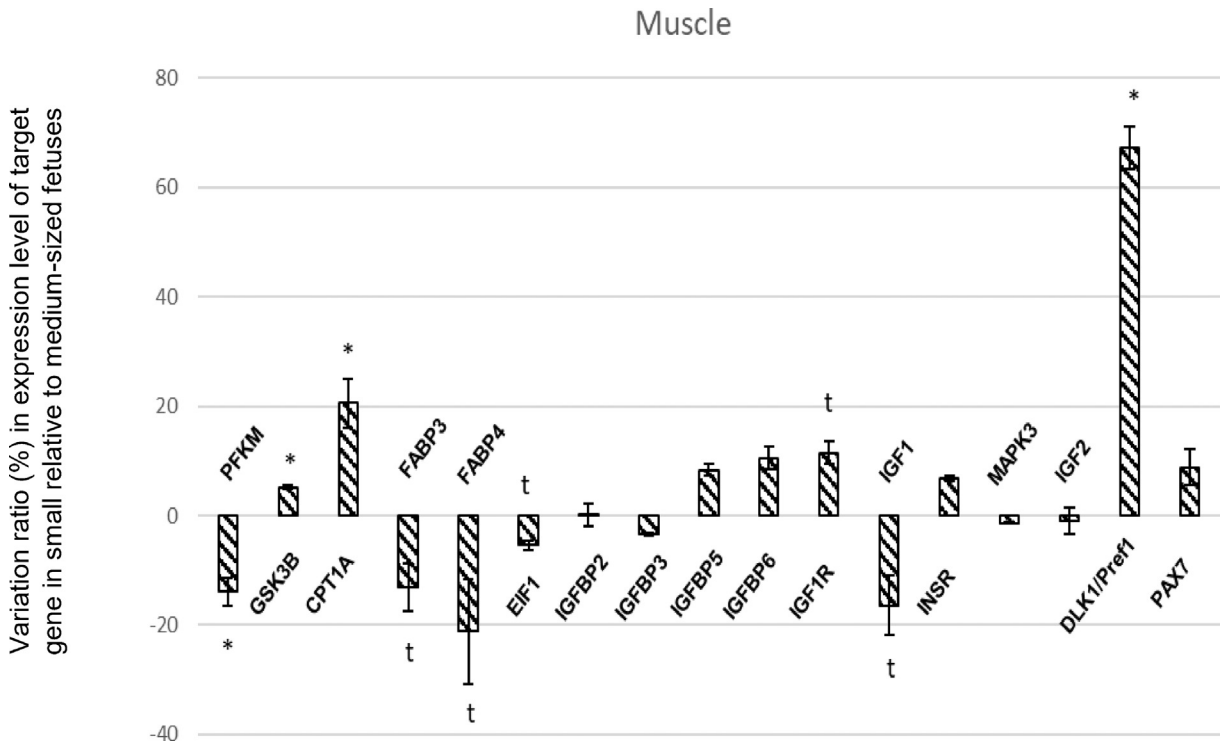


Fig. 3. Muscle expression levels of genes in small- or average-sized pig foetuses. The *Longissimus* muscle was excised from pairs of foetuses (one small, one of average BW) at 110 days of gestation and prepared for molecular analysis. Expression levels of target genes were compared for the effects of maternal treatment, category of foetal BW, and the interaction between maternal treatment and foetal BW category. The %variation ratio (mean + SEM) in expression level of each target gene was shown for small foetuses relative to their average-sized littermates. When a gene was down-regulated by in small foetuses, the value was preceded by a minus sign. $^{*}P \leq 0.05$, $^{t}0.05 < P \leq 0.10$.

immediate prepartum period but it was observed during adverse intrauterine conditions (Fowden et al., 1995). This may explain why genes involved in carbohydrates and lipid metabolism studied in the *Longissimus* muscle had similar expression levels in the two treatment groups. Increased expression levels of genes involved in fatty acid oxidation in the liver of pST foetuses might also prepare the postnatal burst of mitochondrial biogenesis and the shift towards reliance on mitochondrial fatty acid oxidation as the major source of ATP production at birth.

Contrary to fructose, the circulating concentrations of urea were lower in pST foetuses than in control foetuses. These changes paralleled the variations in the maternal blood, with lower urea concentrations measured in pST gilts than in controls at days 96–109 of gestation (Farmer and Langendijk, 2019). Similarly, Gatford et al. (2000) reported that plasma urea concentrations in foetuses decreased with increasing dose of pST administered to sows between 25 and 51 days of gestation. Decreased urea concentrations in pST foetuses may indicate a higher utilisation of protein sources (Rehfeldt et al., 2004). This view is supported by the findings of greater expression levels of *CTSB* and *PSMD1*, two genes involved in the intracellular degradation of proteins, in the liver of pST foetuses compared with controls. The expression level of *GSK3B* in liver was also greater in pST than in control foetuses, which agrees with a higher protein catabolism in the liver of pST foetuses. Indeed, *GSK3B* is involved in targeting substrates for ubiquitin-mediated protein degradation (Verhees et al., 2013) and may regulate the β-catenin activity, an important pathway for hepatocyte proliferation and normal liver growth (Tan et al., 2006). In the current study, there was no effect of treatment on the expression levels of specific genes involved in protein synthesis in the liver so that it remains difficult to give a definitive conclusion on the effect of pST treatment on hepatic protein metabolism.

In *Longissimus* muscle, there were fewer modifications in expression levels of the studied genes in response to maternal pST treatment. The impact of pST on cell development is muscle dependent (Rehfeldt et al., 2001) so that we cannot exclude that more changes have occurred at other muscle locations. Moreover, other pathways may have been used to generate energy in muscle, such as creatine biosynthesis for the production of phosphocreatine and glutaminolysis as a mitochondrial pathway process from the degradation of glutamine. In addition, maternal pST treatment may improve amino acid delivery to the foetus (Gatford et al., 2000) which are themselves key stimulators of the mechanistic target of rapamycin (**mTOR**) pathway, an essential regulator of cell growth and metabolism via the modulation of protein and lipid synthesis (Takahara et al., 2020). Among genes regulating muscle cell growth and development, expression levels of *PAX7* and *MAPK3* were lower in *Longissimus* muscle of pST foetuses than in controls. The *PAX7* gene is usually expressed by quiescent muscle stem cells, which can further enter into the myogenic program. Mitogen-activated protein kinase signalling factors are known to regulate muscle cell differentiation. Among them, *MAPK3* is required for myoblast proliferation and differentiation (Gredinger et al., 1998; Jones et al., 2001). The lower expression level of *PAX7* associated with the lower expression level of *MAPK3* in muscle of pST foetuses may indicate a difference in proliferation ability and(or) a delayed myogenesis (Felicioni et al., 2020) as compared with the control group. A transient delay in muscle differentiation has been noticed in foetuses from sows treated with pST during early gestation (Rehfeldt et al., 2001). In the current study, the expression level of *DLK1*, a gene that is highly expressed in embryonic muscle and is sharply down-regulated during postnatal development in porcine muscles (Oczkowicz et al., 2010), was similar in muscle of pST and of control foetuses. This suggests that there was no or only a moderate delay in muscle differentiation in pST foetuses. Irrespective of maternal treatment, *DLK1* was significantly up-regulated in the muscle of the smallest foetuses, which validates the statistical power of our experimental design to find the

expected difference in expression levels of genes regulating cell development when addressing small- vs medium-sized categories of littermates. Further studies may also consider the ratio of developmental to adult myosin heavy chain isoforms as an additional indicator of muscle maturity degree. Altogether, pST treatment during mid- and late gestation did not induce any changes in the cross-sectional area or total muscle fibre number in neonatal piglets (review by Rehfeldt et al., 2004).

Importantly, nutrient partitioning and utilisation during gestation are under the control of hormones and growth factors, and nutrition may conversely influence the hormonal status. Treatment of pregnant gilts by pST during early gestation altered IGF and IGFBP concentrations in maternal and foetal placental tissues (Freese et al., 2005). In this study, we reported only slight changes associated to pST administration on the insulin-IGF-IGFBP axis in liver and skeletal muscle of pig foetuses. In liver, *IGFBP5* was up-regulated in pST foetuses as compared with controls. Maternal treatment with the phytoestrogen genistein, a type of isoflavone that affects the IGF-I system, increased *IGFBP5* expression in the liver of chick embryos, and this was accompanied by activated apoptosis and protein tyrosine kinase signalling pathways (Lv et al., 2018). Therefore, the greater expression level of *IGFBP5* in liver of pST foetuses matches with the greater expression levels of *CTSD* and *PSMD1* participating in protein catabolism. In muscle, there was a significant down-regulation of *INSR* in pST foetuses, and trends for lower expression levels of *IGF1* and its receptor (*IGF1R)* in this group as compared with controls. Irrespective of maternal treatment, expression level of *IGF1R* was greater in muscle of small foetuses than in their average-sized littermates. This latter finding agrees with other studies showing greater levels of *IGF1R* in *Longissimus* muscle of small foetuses compared with their average-sized littermates (Tilley et al., 2007; Perruchot et al., 2015). Importantly, maternal nutrient restriction in sheep induced an up-regulation of *IGF1R* and *INSR* genes in foetal skeletal muscle, and this was accompanied by a spectral phenotype indicating metabolic programming for developing insulin resistance (Sandoval et al., 2021). Whether the lower expression levels of *INSR*, *IGF1* and *IGF1R* observed herein in foetal muscle in response to maternal pST treatment may lower the risk of metabolic disease for pigs during postnatal period remains to be determined.

Collectively, this study suggests a differential and potentially beneficial ability of the pST-treated sows to support foetal liver development and prepare neonatal metabolism. Nutritional ways to stimulate the GH/IGF-I axis in late gestating gilts should be explored. For example, strategies based on amino acids such as arginine, as well as vitamins and polyphenols, may be promising (Caputo et al., 2001).

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2022.100691.

Ethics approval

Gilts were cared according to a recommended code of practice, and all procedures were approved by the institutional animal care committee of the Sherbrooke Research and Development Centre of Agriculture and Agri-Food Canada (acceptation number 487).

Data and model availability statement

The data that support the study findings are public and available on https://doi.org/10.57745/BDQBF8.

Author ORCIDs

Florence Gondret: https://orcid.org/0000-0001-7997-1560
Isabelle Louveau: https://orcid.org/0000-0001-9684-6294
Chantal Farmer: https://orcid.org/0000-0001-8390-6163

Author contributions

Florence Gondret designed the study on pig fetuses. **Chantal Farmer** conducted the study on sows. **Pieter Langendijk** designed the pST maternal treatment. **Isabelle Louveau** brought her expertise on IGF regulation. **Florence Gondret** analyzed the data and wrote the first draft of the manuscript. All co-authors substantially contributed to the conception or design of the work, critically revised the work and approved the final version. All co-authors agreed to be accountable for all aspects of the work.

Declaration of interest

None.

Acknowledgements

Thanks are due to the technical staff of the Sherbrooke R & D Centre for animal care and sampling, and of INRAE PEGASE (S Tacher, C Tréfeu and S Daré) for expert analyses of tissue traits. We are also most grateful to Biogenouest Genomics, ANAEE-Fr and the EcogenO core facility of Rennes (OSUR) for its technical support.

Financial support statement

The trial on pregnant gilts in this work was financed by Trouw Nutrition (PL and CF). The analyses of molecular traits in foetuses for this work were financed by INRAE (FG and IL).

References

Abd Ellah, N., Taylor, L., Troja, W., Owens, K., Ayres, N., Pauletti, G., Jones, H., 2015. Development of non-viral, trophoblast-specific gene delivery for placental therapy. PLoS One 10, e0140879.

Canario, L., Cantoni, E., Le Bihan, E., Caritez, J.-C., Billon, Y., Bidanel, J.P., Foulley, J.L., 2006. Between-breed variability of stillbirth and its relationship with sow and piglet characteristics. Journal of Animal Science 84, 3185–3196. https://doi.org/10.2527/jas.2005-775.

Canario, L., Père, M.C., Tribout, T., Thomas, F., David, C., Gogué, J., Herpin, P., Bidanel, J.P., Le Dividich, J., 2007. Estimation of genetic trends from 1977 to 1998 of body composition and physiological state of Large White pigs at birth. Animal 1, 1409–1413. https://doi.org/10.1017/S1751731107000766.

Caputo, M., Pigni, S., Agosti, E., Daffara, T., Ferrero, A., Filigheddu, N., Prodam, F., 2001. Regulation of GH and GH Signaling by Nutrients. Cells 10, 1376. https://doi.org/10.3390/cells10061376.

Diggle, C.P., Shires, M., Leitch, D., Brooke, D., Carr, I.M., Markham, A.F., Hayward, B.E., Asipu, A., Bonthron, D.T., 2009. Ketohexokinase: expression and localization of the principal fructose-metabolizing enzyme. Journal of Histochemistry and Cytochemistry 57, 763–774. https://doi.org/10.1369/jhc.2009.953190.

Farmer, C., Langendijk, P., 2019. Exogenous porcine somatotropin stimulates mammary development in late-pregnant gilts. Journal of Animal Science 97, 2433–2440. https://doi.org/10.1093/jas/sks136.

Felicioni, F., Pereira, A.D., Caldeira-Brant, A.L., Santos, T.G., Paula, T.M.D., Magnabosco, D., Bortolozzo, F.P., Tsoi, S., Dyck, M.K., Dixon, W., Martinelli, P. M., Jorge, E.C., Chiarini-Garcia, H., Almeida, F.R.C.L., 2020. Postnatal development of skeletal muscle in pigs with intrauterine growth restriction: morpho-functional phenotype and molecular mechanisms. Journal of Anatomy 236, 840–853. https://doi.org/10.1111/joa.13152.

Fowden, A.L., Apatu, R.S.K., Silver, M., 1995. The glucogenic capacity of the fetal pig: developmental regulation by cortisol. Experimental Physiology 80, 457–467.

Freese, L.G., Rehfeldt, C., Fuerbass, R., Kuhn, G., Okamura, C.S., Ender, K., Grant, A.L., Gerrard, D.E., 2005. Exogenous somatotropin alters IGF axis in porcine endometrium and placenta. Domestic Animal Endocrinology 29, 457–475. https://doi.org/10.1016/j.domaniend.2005.02.012.

Gatford, K.L., Owens, J.A., Campbell, R.G., Boyce, J.M., Grant, P.A., De Blasio, M.J., Owens, P.C., 2000. Treatment of underfed pigs with GH throughout the second

quarter of pregnancy increases fetal growth. Journal of Endocrinology 166, 227–234. https://doi.org/10.1677/joe.0.1660227.
Gondret, F., Père, M.C., Tacher, S., Daré, S., Trefeu, C., Le Huërou-Luron, I., Louveau, I., 2013. Spontaneous intra-uterine growth restriction modulates the endocrine status and the developmental expression of genes in porcine fetal and neonatal adipose tissue. General Comparative Endocrinology 194, 208–216. https://doi.org/10.1016/j.ygcen.2013.09.018.
Gondret, F., Guével, B., Père, M.C., Quesnel, H., Billon, Y., Com, E., Canario, L., Louveau, I., Liaubet, L., 2018. Proteomic analysis of adipose tissue during the last weeks of gestation in pure and crossbred Large White or Meishan fetuses gestated by sows of either breed. Journal of Animal Science Biotechnology 9, 28. https://doi.org/10.1186/s40104-018-0244-2.
Gondret, F., Louveau, I., Langendjik, P., Farmer, C., 2022. O62 Exogenous porcine somatotropin administered to late-pregnant gilts alters hepatic metabolism of protein and energy in pig foetuses. Animal – Science Proceedings 13, 321. https://doi.org/10.1016/j.anscip.2022.07.072.
Gredinger, E., Gerber, A.N., Tamir, Y., Tapscott, S.J., Bengal, E., 1998. Mitogen-activated protein kinase pathway is involved in the differentiation of muscle cells. Journal of Biological Chemistry 273, 10436–10444. https://doi.org/10.1074/jbc.273.17.10436.
Hill, G.M., Mahan, D.C., 2016. Essential and nonessential amino acid compositions of the total litter and individual fetal pig content and accretion rates during fetal development. Journal of Animal Science 94, 5239–5247.
Jones, H.N., Crombleholme, T., Habli, M., 2013. Adenoviral-mediated placental gene transfer of IGF-1 corrects placental insufficiency via enhanced placental glucose transport mechanisms. PLoS One 8, e74632.
Jones, N.C., Fedorov, Y.V., Rosenthal, R.S., Olwin, B.B., 2001. ERK1/2 is required for myoblast proliferation but is dispensable for muscle gene expression and cell fusion. Journal of Cellular Physiology 186, 104–115. https://doi.org/10.1002/1097-4652(200101)186:1.
Kaur, H., Muhlhausler, B.S., Roberts, C.T., Gatford, K.L., 2021. The growth hormone-insulin like growth factor axis in pregnancy. Journal of Endocrinology 251. https://doi.org/10.1530/JOE-21-0087. JOE-21-0087.R1.
Keswani, S.G., Balaji, S., Katz, A.B., King, A., Omar, K., Habli, M., Klanke, C., Crombleholme, T.M., 2015. Intraplacental gene therapy with Ad-IGF-1 corrects naturally occurring rabbit model of intrauterine growth restriction. Human Gene Therapy 26, 172–182. https://doi.org/10.1089/hum.2014.065.
Kveragas, C.L., Seerley, R.W., Martin, R.J., Vandergrift, W.L., 1986. Influence of exogenous growth hormone and gestational diet on sow blood and milk characteristics and on baby pig blood, body composition and performance. Journal of Animal Science 63, 1877–1887.
Leenhouwers, J.I., Knol, E.F., de Groot, P.N., Vos, H., van der Lende, T., 2002. Fetal development in the pig in relation to genetic merit for piglet survival. Journal of Animal Science 80, 1759–1770. https://doi.org/10.2527/2002.8071759x.
Lv, Z., Fan, H., Zhang, B., Ning, C., Xing, K., Guo, Y., 2018. Dietary genistein supplementation in laying broiler breeder hens alters the development and metabolism of offspring embryos as revealed by hepatic transcriptome analysis. FASEB Journal 32, 4214–4228. https://doi.org/10.1096/fj.201701457R.
Noblet, J., Dourmad, J.Y., Etienne, M., 1990. Energy utilization in pregnant and lactating sows: modeling of energy requirements. Journal of Animal Science 68, 562–572.
Oczkowicz, M., Piestrzyska-Kajtoch, A., Piórkowska, K., Rejduch, B., Rózycki, M., 2010. Expression of DLK1 and MEG3 genes in porcine tissues during postnatal development. Genetics and Molecular Biology 33, 790–794. https://doi.org/10.1590/s1415-47572010000400030.
Perruchot, M.H., Lefaucheur, L., Louveau, I., Mobuchon, L., Palin, M.F., Farmer, C., Gondret, F., 2015. Delayed muscle development in small pig fetuses around birth cannot be rectified by maternal early feed restriction and subsequent overfeeding during gestation. Animal 9, 1996–2005. https://doi.org/10.1017/S1751731115001202.
Puigserver, P., Spiegelman, B.M., 2003. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. Endocrinology Review 24, 78–90. https://doi.org/10.1210/er.2002-0012.
Quesnel, H., Brossard, L., Valancogne, A., Quiniou, N., 2008. Influence of some sow characteristics on within-litter variation of piglet birth weight. Animal 2, 1842–1849. https://doi.org/10.1017/S175173110800308X.
Rehfeldt, C., Kuhn, G., Vanselow, J., Fürbass, R., Fiedler, I., Nürnberg, G., Clelland, A.K., Stickland, N.C., Ender, K., 2001. Maternal treatment with somatotropin during early gestation affects basic events of myogenesis in pigs. Cell Tissue Research 306, 429–440. https://doi.org/10.1007/s00441-001-0475-x.
Rehfeldt, C., Nissen, P.M., Kuhn, G., Vestergaard, M., Ender, K., Oksbjerg, N., 2004. Effects of maternal nutrition and porcine growth hormone (pGH) treatment during gestation on endocrine and metabolic factors in sows, fetuses and pigs, skeletal muscle development, and postnatal growth. Domestic Animal Endocrinology 27, 267–285. https://doi.org/10.1016/j.domaniend.2004.06.005.
Sandoval, C., Askelson, K., Lambo, C.A., Dunlap, K.A., Satterfield, M.C., 2021. Effect of maternal nutrient restriction on expression of glucose transporters (SLC2A4 and SLC2A1) and insulin signaling in skeletal muscle of SGA and Non-SGA sheep fetuses. Domestic Animal Endocrinology 74,. https://doi.org/10.1016/j.domaniend.2020.106556 106556.
Sferruzzi-Perri, A.N., Owens, J.A., Pringle, K.G., Robinson, J.S., Roberts, C.T., 2006. Maternal insulin-like growth factors-I and -II act via different pathways to promote fetal growth. Endocrinology 147, 3344–3355. https://doi.org/10.1210/en.2005-1328.
Takahara, T., Amemiya, Y., Sugiyama, R., Maki, M., Shibata, H., 2020. Amino acid-dependent control of mTORC1 signaling: a variety of regulatory modes. Journal of Biomedicine Science 27, 87. https://doi.org/10.1186/s12929-020-00679-2.
Tan, X., Behari, J., Cieply, B., Michalopoulos, G.K., Monga, S.P., 2006. Conditional deletion of beta-catenin reveals its role in liver growth and regeneration. Gastroenterology 131, 1561–1572. https://doi.org/10.1053/j.gastro.2006.08.042.
Thorn, S.R., Rozance, P.J., Brown, L.D., Hay Jr., W.W., 2011. The intrauterine growth restriction phenotype: fetal adaptations and potential implications for later life insulin resistance and diabetes. Seminars in Reproduction Medicine 29, 225–236. https://doi.org/10.1055/s-0031-1275516.
Tilley, R.E., McNeil, C.J., Ashworth, C.J., Page, K.R., McArdle, H.J., 2007. Altered muscle development and expression of the insulin-like growth factor system in growth retarded fetal pigs. Domestic Animal Endocrinology 32, 167–177. https://doi.org/10.1016/j.domaniend.2006.02.003.
Towler, M.C., Hardie, D.G., 2007. AMP-activated protein kinase in metabolic control and insulin signaling. Circulation Research 100, 328–341. https://doi.org/10.1161/01.RES.0000256090.42690.05.
Vallet, J.L., McNeel, A.K., Miles, J.R., Freking, B.A., 2014. Placental accommodations for transport and metabolism during intra-uterine crowding in pigs. Journal of Animal Science and Biotechnology 5, 55. https://doi.org/10.1186/2049-1891-5-55.
Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., Speleman, F., 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biology 3. https://doi.org/10.1186/gb-2002-3-7-research0034. RESEARCH0034.
Verhees, K.J., Pansters, N.A., Schols, A.M., Langen, R.C., 2013. Regulation of skeletal muscle plasticity by glycogen synthase kinase-3β: a potential target for the treatment of muscle wasting. Current Pharmaceutical Design 19, 3276–3298. https://doi.org/10.2174/1381612811319180011.
Vincent, A., Louveau, I., Gondret, F., Tréfeu, C., Gilbert, H., Lefaucheur, L., 2015. Divergent selection for residual feed intake affects the transcriptomic and proteomic profiles of pig skeletal muscle. Journal of Animal Science 93, 2745–2758. https://doi.org/10.2527/jas.2015-8928.